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14. ABSTRACT

Enter a brief (approximately 200 words) We hypothesized that dehydroepiandrosterone metabolites or their synthetic derivatives are able to bind to the androgen receptor with low, if any, agonist activity and thus function as better antiandrogens than currently available ones. We previously identified three potential compounds with marginal androgenic activity. Using different prostate cancer cell lines, we evaluated the effects of these steroids on cell proliferation/apoptosis, cell migration/invasion, and the expression of several molecules related to cell growth/angiogenesis/metastasis. We found that these compounds indeed exhibited antiandrogenic activities, particularly on cell proliferation/apoptosis and prostate-specific antigen expression, although they were not always significant. Our results suggest that these compounds are superior to current antiandrogens, in terms of androgenic and antiandrogenic properties in prostate cancer cells in vitro. Tolerance of these compounds was also assessed in mice. The animals seemed to suffer from no adverse effects from injections of the steroids. We will next evaluate the anti-tumor effects of these compounds in mouse xenograft models for prostate cancer.

15. SUBJECT TERMS

Antiandrogen, androgen receptor, dehydroepiandrosterone, cell growth, tolerance

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Introduction

Although antiandrogens that can block androgen action through the androgen receptor (AR) have been widely used for the treatment of prostate cancer, the majority of available ones possess agonist activity, resulting in increases in serum prostate-specific antigen (PSA) levels, known as the antiandrogen withdrawal syndrome [1,2]. In addition, we previously found that androstenediol (Adiol), a physiological metabolite from dehydroepiandrosterone (DHEA) and a precursor of testosterone, has an intrinsic androgenic activity which was not completely antagonized by two clinically used antiandrogens, hydroxyflutamide (HF) and bicalutamide (BC) [3]. Therefore, new and more effective antiandrogenic compounds with marginal androgenic activities need to be identified. Our hypothesis in the current project was that DHEA metabolites or their synthetic derivatives are able to bind to the AR with low, if any, agonist activity and thus function as better antiandrogens than currently available ones. We previously screened DHEA derivatives/metabolites for their androgenic and antiandrogenic activities and found that three compounds, 3β-acetoxyandrost-1,5-diene-17-ethylene-ketal (ADEK), 3β-hydroxyandrost-5,16-diene (HAD), and 3-oxo-androst-1,4-diene-17-ketal (OADK), show only marginal agonist effects and suppress significantly 5α-dihydrotestosterone (DHT)- and Adiol-induced AR transactivations [4-6]. Thus, ADEK, HAD, and OADK have the potential to function as potent antiandrogens that carry fewer risks of withdrawal response if used for therapy in patients with prostate cancer.

The tasks in the approved Statement of Work in this period (months 13-24) would be to evaluate the effects of DHEA derivatives *in vitro* (*Task 1* for months 1-18), including *Task 1-a* (to test the effects of the compounds on prostate cancer cell proliferation, apoptosis, cell invasion, expression of PSA and other genes) and *Task 1-b* (to test the effects of long-term treatment with the compounds on prostate cancer cell growth), and to evaluate the effects of DHEA derivatives *in vivo* (*Task 2* for months 19-48), including *Task 2-a* (to test the tolerance and toxicity of the compounds in mice).

Body

Effects of ADEK, HAD, and OADK on cell proliferation. Using MTT (thiazolyl blue) assay, we first examined androgenic/antiandrogenic effects of ADEK, HAD, and OADK, in comparison with those of HF, on cell proliferation of six prostate cancer cell lines with different AR statuses. LNCaP and CWR22Rv1 express a mutant AR T877A and a mutant AR H874Y, respectively. PC-3(AR)2 and PC-3(AR)9 are stable clones of AR-negative PC-3 with wild-type AR under control of a cytomegalovirus promoter [7] and a natural AR promoter [6,8], respectively. The cell lines were cultured for 6 days in the presence or absence of 1 nM DHT and different concentrations of HF, ADEK, HAD, or OADK. In LNCaP, DHT or HF increased cell growth by nearly 100% after 6-day culture (Figure 1A, lanes 1 *vs.* 2 or 9), whereas ADEK, HAD, and OADK, except 1 μM OADK, showed marginal (<10%) growth induction in the absence of androgens (lanes 1 *vs.* 3-8). ADEK (0.1 and 1 μM), HAD (1 μM), and OADK (0.1 μM), but not HF, significantly antagonized the effect of DHT (lanes 9 *vs.* 10-16). Similarly, in CWR22Rv1, HF, ADEK,

HAD, and OADK, except 1 μM OADK, showed marginal (<10%) growth induction in the absence of androgens (Figure 1B, lanes 1 *vs.* 2-8). HF (1 μM), ADEK (0.1 and 1 μM), HAD (1 μM), and OADK (0.1 μM) significantly antagonized the DHT effect (lanes 9 *vs.* 10-16). In PC-3(AR)9, DHT increased cell growth by only 13% (Figure 1D, lanes 1 *vs.* 9). Although HF, ADEK, HAD, and OADK showed marginal (<7%) growth induction in the absence of androgens (lanes 1 *vs.* 2-8), these compounds did not significantly antagonize the effect of DHT (<6%; lanes 9 *vs.* 10-16). In PC-3(AR)2 (Figure 1C), PC-3 (Figure 1E), and DU145 (Figure 1F), DHT, HF, and/or each of the 3 steroid derivatives showed little effects on cell growth. We also performed MTT assay in the same cell lines with treatment of Adiol (instead of DHT) and the antiandrogenic compounds. However, up to 10 nM of Adiol (physiological concentrations in men are ~5 nM [3]) did not significantly increase the growth of any of the six cell lines, and, therefore, only marginal suppression by steroid derivatives were seen.

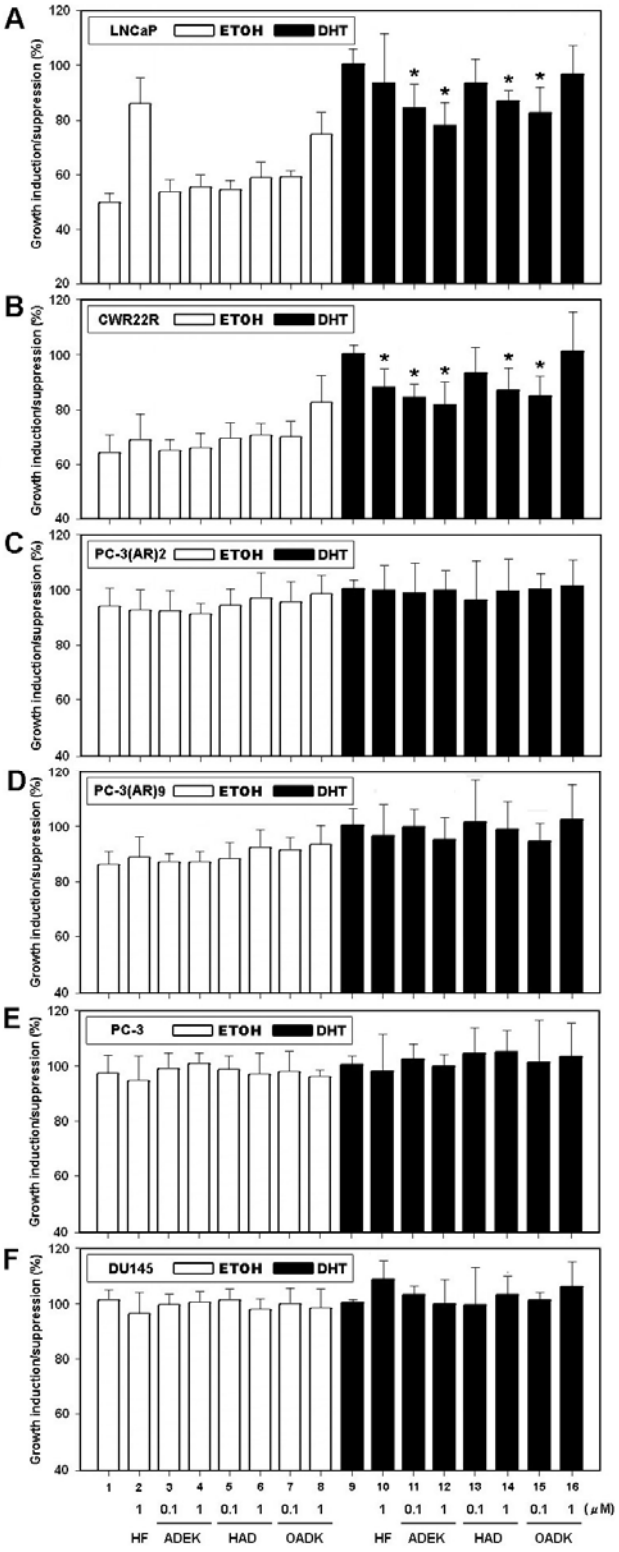


Figure 1. The effects of DHEA derivatives on cell proliferation. LNCaP (A), CWR22Rv1 (B), PC-3(AR)2 (C), PC-3(AR)9 (D), PC-3 (E), or DU145 (F) cells were cultured for 6 days with different concentrations of HF, ADEK, HAD, OADK in the absence [ethanol (ETOH); white bars] or presence (black bars) of 1 nM DHT, as indicated. The MTT assay was performed and growth induction/ suppression is presented relative to cell number with DHT treatment in each panel (ninth lanes; set as 100%). Values represent the mean ± SD of at least three determinations. *p<0.05 (*vs.* DHT for lanes 10-16; analyzed by Student's *t*-test).

Anti-DHT effects of ADEK, HAD, and OADK on apoptosis. Using DNA fragmentation (TUNEL) assay, we next assessed antiandrogenic effects of ADEK, HAD, and OADK on apoptosis. Prostate cancer cell lines were cultured for 6 days with 1 nM DHT and different concentrations of HF (1 μM only), ADEK, HAD, or OADK. Apoptotic indices

were determined by fluorescence microscopy. As summarized in Table 1, ADEK (0.1, 1 μM), HAD (1 μM), and OADK (0.1, 1 μM), in the presence of DHT (1 nM), were found to induce apoptosis in LNCaP and CWR22Rv1 cells. In contrast, in PC-3 as well as PC-3(AR)2 and PC-3(AR)9 cells, there are no significant differences in apoptotic indexes between DHT with and without each antiandrogenic compound.

Table 1. Apoptosis in prostate cancer cell lines following the treatment of DHEA derivatives.

Treatment (+ 1 nM DHT)	LNCaP	CWR22R	PC-3(AR)2	PC-3(AR)9	PC-3
Mock (ETOH)	1.6	2.7	5.0	3.9	1.8
HF (1 μM)	2.4	9.2	5.5	5.1	2.2
ADEK (0.1 μM)	12.8	16.4	5.8	6.9	1.9
ADEK (1 μM)	22.3	20.6	6.3	7.7	1.9
HAD (0.1 μM)	4.9	5.4	5.8	6.0	1.8
HAD (1 μM)	18.8	16.6	5.7	7.0	2.2
OADK (0.1 μM)	19.3	20.4	6.9	9.5	2.7
OADK (1 μM)	17.8	18.5	6.2	9.9	1.9

Apoptotic index = percentage of TUNEL-positive cells in a total of 1,000 cells.

Anti-DHT effects of ADEK, HAD, and OADK on colony formation. We determined the cell survival in a colony formation assay. The number of colonies formed in LNCaP (Figure 2A) or CWR22Rv1 (Figure 2B) cells was significantly augmented by DHT treatment. However, the DHEA derivatives as well as HF showed marginal to only slight reductions in LNCaP (up to 13%) and CWR22Rv1 (up to 12%). These results suggest that antiandrogens tested do not significantly inhibit androgen-induced colony formation of prostate cancer cells.

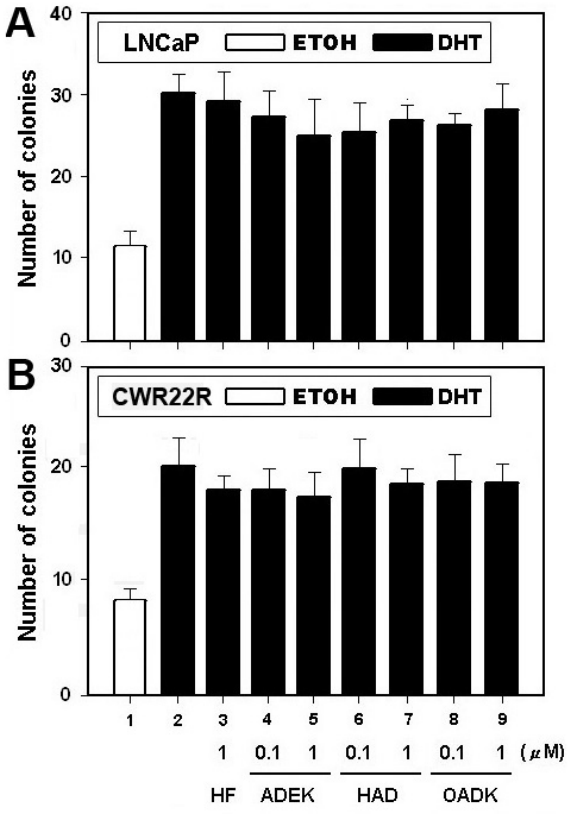


Figure 2. The effects of DHEA derivatives on colony-forming. LNCaP (A) or CWR22Rv1 (B) cells plated onto the soft agar were cultured for 2 weeks with different concentrations of HF, ADEK, HAD, OADK in the absence [ethanol (ETOH); white bar] or presence (black bars) of 1 nM DHT, as indicated, and stained with methylene blue. Colonies with cell numbers higher than 50 were counted. Values represent the mean ± SD of at least three determinations.

Anti-DHT effects of ADEK, HAD, and OADK on cell migration and invasion. We determined the invasion ability of prostate cancer cells using the transwell chamber assay. As shown in Figures 3A and 3B, DHT enhanced the invasion ability in LNCaP (40% increase) and CWR22Rv1 (59% increase) cells, respectively. However, the DHEA derivatives and HF showed marginal to only slight reductions in the presence of DHT. In PC-3(AR)2, PC-3(AR)9 (Figure 3C), or PC-3 cells, DHT marginally (up to 16%) increased the invasion, and DHEA derivatives did not show significant inhibitory effects. These results suggest that antiandrogens tested do not significantly inhibit androgen-induced cell migration and invasion of prostate cancer cells.

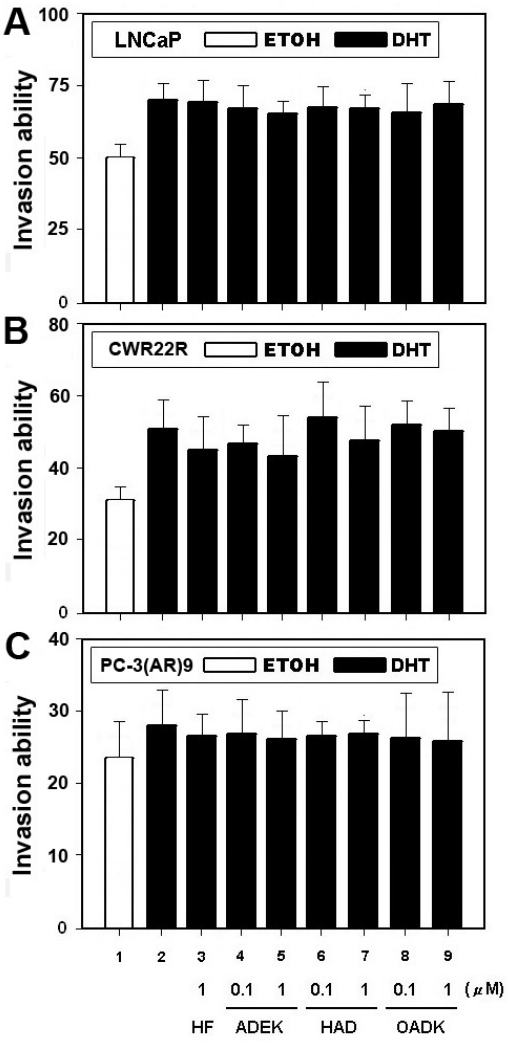


Figure 3. The effects of DHEA derivatives on invasive ability. Matrigel in serum-free cold cell culture medium was placed in the upper chamber of a 24-well transwell and incubated for 5 h at 37°C. LNCaP (A), CWR22Rv1, or PC-3(AR)9 (C) cells were harvested, and cell suspensions (100 μl) were placed on the matrigel, and the lower chamber of the transwell was filled with culture medium in the presence of 5 μg/ml fibronectin, as an adhesive substrate. DHT (1 nM) together with different concentrations of HF, ADEK, HAD, or OADK was added in both upper and lower chambers. Following 48 h of incubation at 37°C, transwells were removed and stained with Giemsa solution. Non-invading cells on top of transwells were removed, and invading cells were counted under the microscope. Values represent the mean ± SD of at least three determinations.

Anti-DHT effects of ADEK, HAD, and OADK on mRNA expression of PSA, AR, and other molecules related to angiogenesis and metastasis. A quantitative reverse transcription (RT)-polymerase chain reaction (PCR) analysis was then performed in AR-positive/PSA-positive prostate cancer cells, LNCaP and CWR22Rv1, in order to assess the antiandrogenic effects of ADEK, HAD, and OADK on tumor progression (*i.e.* PSA, AR) and angiogenesis/metastasis [*i.e.* basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), interleukin (IL)-6, and matrix metalloproteinase (MMP)-9]. As expected, DHT increased and ADEK (1 μM), HAD (1 μM), and OADK (0.1 μM) suppressed DHT-enhanced PSA expression both in LNCaP and CWR22Rv1 cells (Figure 4A). DHT increased AR mRNA, and only ADEK (in LNCaP) or HAD (in CWR22Rv1) significantly inhibited the DHT-mediated AR expression, although all 3 compounds, as well as HF, showed a tendency to decrease it (Figure 4B). Similarly, DHT could significantly increase the expression of bFGF (Figure 4C), VEGF (Figure 4D), IL-6 (Figure 4E), and MMP-9 (Figure 4F) in LNCaP and CWR22Rv1 cells. However, only some treatments significantly antagonized the effects of DHT (ADEK/HAD for VEGF in LNCaP, HAD for VEGF in CWR22Rv1, and ADEK for IL-6 or

MMP-9 in CWR22Rv1). Again, 10 nM Adiol did not significantly increase the expression of the molecules, which was only marginally suppressed by the steroid derivatives.

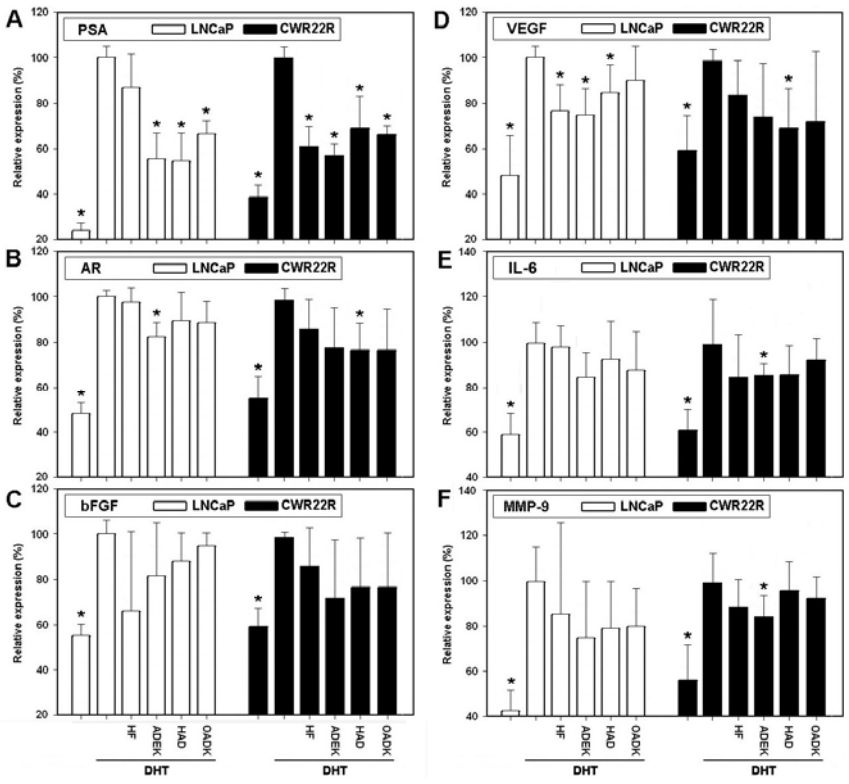
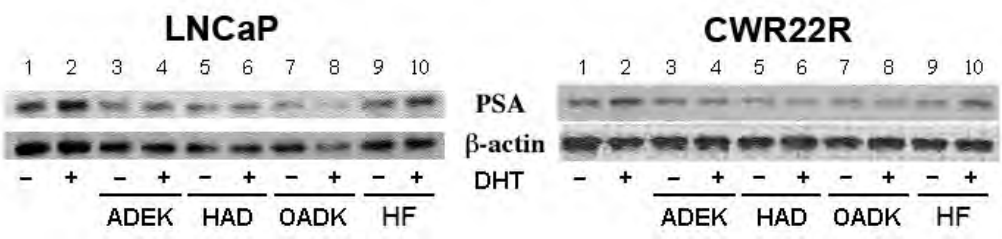


Figure 4. The effects of DHEA derivatives on expression of PSA (A), AR (B), bFGF (C), VEGF (D), IL-6 (E), and MMP-9 (F). LNCaP (white bars) or CWR22Rv1 (black bars) cells were cultured for 48 h with 1 μM HF, 1 μM ADEK, or 1 μM HAD, or 0.1 μM OADK in the absence or presence of 1 nM DHT, as indicated. Total RNAs from these cells were isolated and reverse transcribed. Real-time PCR was then performed, using each specific primer set. GAPDH was used as an internal control. Expression levels are presented relative to those with DHT treatment in each panel (second lanes; set as 100%). Values represent the mean ± SD of at least three determinations. *p<0.05 (*vs.* DHT; analyzed by Student's *t*-test).

Anti-DHT effects of ADEK, HAD, and OADK on protein expression of PSA. Western blotting analysis was performed, using a PSA antibody (DAKO), as described previously [5,6], in order to determine whether DHEA derivatives inhibit androgen-mediated PSA protein expression in prostate cancer cells. As expected, DHT increased endogenous PSA expression in LNCaP or CWR22Rv1 cells over mock treatment (Figure 5, lanes 1 *vs.* 2). ADEK, HAD, and OADK showed only marginal induction without androgens (lanes 1 *vs.* 3, 5, or 7) and antagonized DHT-induced PSA expression (lanes 2 *vs.* 4, 6, or 8). HF did not show significant anti-DHT effects (lanes 2 *vs.* 10).

Figure 5. The effects of DHEA derivatives on PSA expression. Cell extracts from LNCaP or CWR22Rv1 cultured for 48 h with ADEK (1 μM), HAD (1 μM), OADK (0.1 μM), or HF (1 μM) in the absence or presence of 1 nM DHT, as indicated, were analyzed on Western bots, using an antibody to PSA (upper) or β-actin (lower). The 33 kDa (for PSA) and 43 kDa (for β-actin as an internal control) proteins were detected.



Anti-DHT effects of ADEK, HAD, and OADK on protein expression of AR and other molecules related to angiogenesis and metastasis. Additional Western blotting analyses were performed to assess whether DHEA derivatives inhibit androgen-induced protein expression of AR and angiogenic/metastatic factors in prostate cancer cells. Such factors included VEGF, IL-6, and MMP-9. As expected, DHT increased endogenous expression of AR and other factors in LNCaP or CWR22Rv1 cells over mock treatment (Figure 6). Then, ADEK, HAD, and OADK showed antiandrogenic effects on the expression of most of the proteins without significant agonist activities.

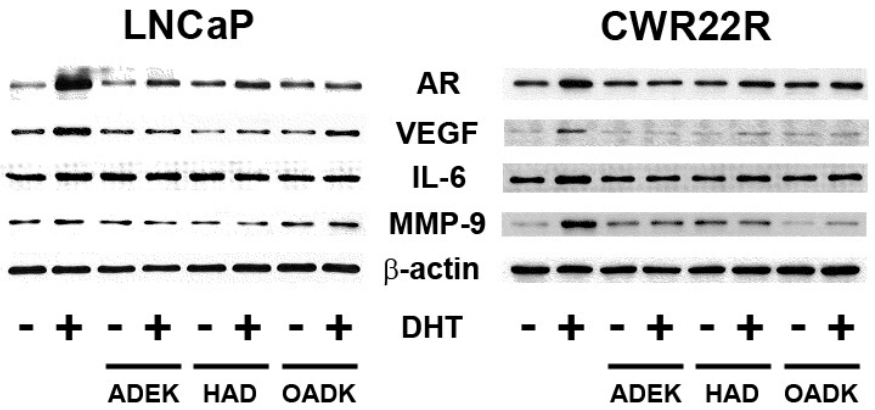


Figure 6. The effects of DHEA derivatives on the expression of proteins related to angiogenesis/ metastasis. Cell extracts from LNCaP or CWR22Rv1 cultured for 48 h with ADEK (1 μM), HAD (1 μM), or OADK (0.1 μM) in the absence or presence of 1 nM DHT, as indicated, were analyzed on Western bots, using an antibody to AR (N20), VEGF, IL-6, MMP-9, or β-actin (all from Santa Cruz Biotechnology).

Effects of long-term treatment with ADEK, HAD, or OADK. To determine whether long-term cultures with each DHEA derivative lead to any changes in the cells (*e.g.* growth rate, expression of AR and PSA, response to androgen supplementation), we have cultured LNCaP cells for at least 20 weeks with 1 μM ADEK, 1 μM HAD, or 1 μM OADK. Using these sublines, we then performed MTT assay, RT-PCR, and Western blotting, as described above. Unfortunately, we identified no significant differences in cell growth in the presence or absence of androgen and the expression of androgen-regulated proteins between each subline versus control subline. We continue to culture the sublines in the same conditions. In addition, we will culture LNCaP cells with higher concentrations of each DHEA derivative.

Tolerance/toxicity for ADEK, HAD, and OADK in animals. To determine whether the DHEA derivatives are well tolerated or affected any adverse responses in animals, ADEK, HAD, or OADK (200 mg/Kg daily for 14 days; 2x postulated therapeutic dose [9]) was administered subcutaneously in 6-week-old male C57BL/6 mice (n = 5 / each group). The data in Table 2 demonstrate that there were no statistically significant differences (by Student's *t*-test) in food intake, weight gain, and weights of the heart, liver, kidney, adrenal, spleen, testis, and brain between control and treatment groups. These organs were also histologically examined. I, as a certified pathologist, confirmed that there were no significant visible changes in the H&E stained tissues from different groups of mice. Thus, it was likely that animals with injections of a high dose of ADEK, HAD, or OADK suffered from no adverse effects.

Table 2. Responses to injected DHEA derivatives in mice.

	Control	ADEK	HAD	OADK
Weight gain (g)	1.7	1.5	1.6	1.6
Food intake (g)	55.8	52.1	52.7	55.0
Heart (mg)	120	109	111	118
Liver (mg)	1,180	1,221	1,215	1,264
Kidney (R+L, mg)	387	402	398	380
Adrenal (R+L, mg)	12	11	10	11
Spleen (mg)	78	74	77	81
Testis (R+L, mg)	365	339	342	351
Brain (mg)	416	423	424	410

The data represent the average weights from 5 mice.

Key Research Accomplishments

1. (for *Task 1-a*) Using different prostate cancer cell lines, we demonstrated antiandrogenic effects of ADEK, HAD, and OADK on cell proliferation/apoptosis, colony formation, cell migration/invasion, and the expression of AR, PSA, and angiogenic/metastatic factors, with marginal androgenic activities, *in vitro* (Figures 1-6 and Table 1).

2. (for *Task 1-b*) There were no significant differences in cell growth or the expression of androgen-regulated proteins among LNCaP sublines after long-term culture (at least 20 weeks) with ADEK, HAD, OADK, or BC.

3. (for *Task 2-a*) ADEK, HAD, and OADK were well tolerated or affected no adverse responses in mice.

Reportable Outcomes

Miyamoto H, Lai K-P, Zheng Y, Izumi K, Chang C: Identification of steroid derivatives as potent androgen receptor antagonists with marginal androgenic and estrogenic activity. Presented in IMPaCT2011 at Orlando, Florida (3/10/2011).

(Publications)
 # Indicates the acknowledgment of the current award in the published article.

Miyamoto H, Brimo F, Schultz L, Ye H, Miller JS, Fajardo DA, Lee TK, Epstein JI, Netto GJ: Low-grade papillary urothelial carcinoma of the urinary bladder: A clinicopathologic

analysis of a post-World Health Organization/International Society of Urological Pathology classification cohort from a single academic center. *Arch Pathol Lab Med* 134(8): 1160-1163, 2010 (August).

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Mir C, Shariat SF, van der Kwast TH, Ashfaq R, Lotan Y, Evans A, Skeldon S, Hanna S, Vajpeyi R, Kuk C, Alkhateeb S, Morote J, van Rhijn BWG, Bostrom P, Yao J, **Miyamoto H**, Jewett M, Fleshner N, Messing E, Zlotta AR: Loss of androgen receptor expression is not associated with pathological grade, stage, gender or outcome in bladder cancer: a large multi-institutional study. *BJU Int* 108(1): 24-30, 2011.

Canacci AM, Izumi K, Zheng Y, Gordetsky J, Yao JL, **Miyamoto H** (corresponding author): Expression of semenogelins I and II and its prognostic significance in human prostate cancer. *Prostate* 71(10): 1108-1114, 2011.#

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Gordetsky J, Findeis-Hosey J, Erturk E, Messing EM, Yao JL, **Miyamoto H** (corresponding author): Intraoperative frozen section analysis in fibrous pseudotumor of the testicle: a five-case experience. *Can Urol Assoc J (CUAJ)*, in press (January 2011).

Lee TK, Chaux A, Karram S, **Miyamoto H**, Miller JS, Fajardo DA, Epstein JI, Netto GJ: Papillary urothelial neoplasm of low malignant potential of the urinary bladder: Clinicopathologic and outcome analysis from a single academic center. *Hum Pathol*, in press (March 2011).

Fajardo DA, **Miyamoto H**, Miller JS, Lee TK, Epstein JI: Identification of Gleason pattern 5 on prostatic needle core biopsy: Frequency of underdiagnosis and relation to morphology. *Am J Surg Pathol*, in press (May 2011).

Miyamoto H (corresponding author), Yao JL, Chaux AM, Zheng Y, Hsu I, Izumi K, Chang C, Messing EM, Netto GJ, Yeh S: Expression of androgen and estrogen receptors and its prognostic significance in urothelial neoplasm of the urinary bladder. *BJU Int*, in press (June 2011).

Miyamoto H: "Immunocytochemistry and non-cytologic methods for detection of urothelial neoplasm" in Koss's Cytology of Lower Urinary Tract with Histopathologic Correlations (Koss LG/Hoda RS Eds.), Humana Press, New York (ISBN 978-1-60327-446-3), in press (June 2011).

(Other Presentations/Abstracts)

Canacci AM, Izumi K, Zheng Y, Gordetsky J, Yao JL, **Miyamoto H**: Expression of seminal plasma proteins in prostate cancer: Prognostic implications after radical prostatectomy. 100th Annual Meeting United States & Canadian Academy of Pathology at San Antonio, Texas (2/28/2011); Abstract published in *Mod Pathol* 24(Suppl 1): 182A, 2011.

Subik MK, Yao JL, di Sant'Agnese PA, **Miyamoto H**: The role of periprostatic and periseminal vesicle lymph node metastasis in the staging and prognosis of prostate cancer. 100th Annual Meeting United States & Canadian Academy of Pathology at San Antonio, Texas (2/28/2011); Abstract published in *Mod Pathol* 24(Suppl 1): 226A, 2011.

Subik MK, Gordetsky J, Yao JL, di Sant'Agnese PA, **Miyamoto H**: Frozen section assessment in testicular and paratesticular lesions suspicious for malignancy: its role in preventing unnecessary orchiectomy. 100th Annual Meeting United States & Canadian Academy of Pathology at San Antonio, Texas (2/28/2011); Abstract published in *Mod Pathol* 24(Suppl 1): 226A, 2011.

Yeh S, **Miyamoto H**: Loss of tocopherol associated protein stimulates prostate cancer growth and is associated with metastasis. IMPaCT2011 at Orlando, Florida (3/10/2011).

Miyamoto H, Yao JL, Zheng Y, Hsu I, Izumi K, Netto GJ, Messing EM, Chang C, Yeh S: Expression of androgen and estrogen receptors and its prognostic significance in high-grade urothelial carcinoma of the bladder. American Urological Association 106th Annual Meeting at Washington, DC (5/15/2011); Abstract published in *J Urol* 185(4 Suppl): e121, 2011.

Zheng Y, Izumi K, Yao JL, **Miyamoto H**: Androgen up-regulates the expression of EGFR and ERBB2 in bladder cancer cells. American Urological Association 106th Annual Meeting at Washington, DC (5/16/2011); Abstract published in *J Urol* 185(4 Suppl): e424-e425, 2011.

Izumi K, Zheng Y, **Miyamoto H**: Androgen receptor signals regulate UDP-glucuronosyltransferases: A potential mechanism of androgen-induced bladder carcinogenesis. American Urological Association 106th Annual Meeting at Washington, DC (5/16/2011); *The Journal of Urology* 185(4 Suppl): e430-e431, 2011.

Izumi K, Canacci AM, Zheng Y, Gordetsky J, Yao JL, **Miyamoto H**: Expression of seminal plasma proteins in prostate cancer: Prognostic implications after radical prostatectomy. American Urological Association 106th Annual Meeting at Washington, DC (5/18/2011); Abstract published in *J Urol* 185(4 Suppl): e936, 2011.

Hsu J-W, **Miyamoto H**, Chang C: Decreased tumorigenesis, growth, and mortality of chemical carcinogen-induced bladder cancer in mice lacking urothelial androgen

receptor. The 93rd Annual Meeting of The Endocrine Society at Boston, MA (June 2011); Abstract published in *Endocr Rev* 32(3 Suppl): P3-31, 2011.

(Invited Speakers)

Department of Urology Grand Rounds, SUNY Upstate Medical University, Syracuse, NY (12/2/2010).

Department of Urology Grand Rounds, University of Rochester Medical Center, Rochester, NY (3/16/2011).

Conclusion

Using the prostate cancer cell lines with different AR statuses, androgenic/antiandrogenic effects of ADEK, HAD, and OADK on cell proliferation/apoptosis and the expression of AR, PSA, and angiogenesis/metastasis-related molecules were evaluated. We found that these compounds indeed exhibited antiandrogenic activities, although they were not always significant. These compounds showed marginal effects on androgen-induced colony formation and cell migration/invasion. Nonetheless, our results suggest that ADEK, HAD, and OADK are superior to HF, in terms of androgenic and antiandrogenic properties in prostate cancer cells *in vitro*. We also tested the tolerance and toxicity of ADEK, HAD, and OADK in mice and identified no adverse effects. We are ready to perform further *in vivo* studies to assess the anti-tumor effects of these compounds in mouse xenograft models for prostate cancer.

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Appendices

Not applicable.